*On the Absorption of Agglutinin by Bacteria and the Application of Physico-chemical Laws thereto.**

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Eisenberg and Volk, in 1902, were the first to endeavour to make quantitative measurements of the absorption of agglutinins by bacteria. They showed that if an agglutinating serum in varying dilution was treated with a constant amount of the homologous bacteria, the amount of agglutinin absorbed by the bacteria was not constant. In a concentrated serum the absolute amount absorbed was greater than when the same serum was used after dilution, whilst, on the other hand, the relative amount absorbed from the concentrated serum was less. Hitherto these experiments have been regarded as the fundamental groundwork for the whole discussion on the combination between agglutinins and bacteria.

Arrhenius was the first who tried to apply the laws of physical chemistry to the question of immunity, stating that the interaction between toxins and antitoxins, explained by Ehrlich as complex, was in reality relatively simple. He stated that the combination of a toxin with its antitoxin resembled that of a weak acid—boracic for example—with ammonia, and that the combinations into which the bacterial toxins entered could be explained by the simple laws holding good in the interactions of simple chemical compounds, and without having recourse to the very complicated structures assigned by Ehrlich to diphtheria toxin or tetanus toxin, etc. These theories were mainly based on experiments carried out by Madsen. Arrhenius, from the beginning, has considered the absorption of an agglutinin by its corresponding bacteria as being the most simple one in the domain of immunity, and as being entirely different to the interaction between toxins and their antitoxins.

* The experiments in this paper were partly carried out in the University Laboratory for Medical Bacteriology, Copenhagen, and we wish to express our gratitude to Prof. Salomonsen, the Director of that laboratory, for the great facilities he always granted us while we worked there.

Before describing our own results, we shall briefly mention Arrhenius' view as to the absorption of agglutinins by bacteria. Taking the experiments of Eisenberg and Volk, he showed that there existed a relation between the quantity of absorbed agglutinin (C) and the free agglutinin (B), as expressed by the formula $C = kB^{\frac{2}{3}}$, where k is a constant.

"The physical interpretation of the above formula is very simple. It states that the agglutinin molecules are divided between two solvents, the bacterial cells and the surrounding medium, and that of two molecules of the free agglutinin are formed three molecules of the absorbed agglutinin" (Arrhenius). This is a special case of the Guldberg-Waage law of chemical mass action, and is comparable to the distribution of benzoic acid in the two different solvents, water and benzene, where the concentration of the aqueous solution (C_a) is related to the concentration of the benzene solution (C_b), according to the formula $C_a = kC_b^{\frac{1}{2}}$, as has been shown by Nernst.

Later on, Arrhenius changed his formula from $C = kB^{\frac{2}{3}}$ to $C = kB^n$, as it was found from a series of preliminary experiments carried out by one of us (G. D.), but hitherto not published, that not only the constant k, but also the exponent n varied in different experiments, this variation in the exponent n changing the interpretation of the formula from a simple to an elaborate one.

Arrhenius states in his book on Immuno-Chemistry that n, in the case of the absorption of agglutinin by bacteria, always falls near unity, which is certainly not the case, and he also states that, as n may even be greater than unity, its value has a certain theoretical significance, as an aid in deciding the nature of the process involved in the absorption of the agglutinin.

In support of his theory dealing with equilibria in absorption processes, Arrhenius brings forward the following principal arguments :—

1. That the absorption of agglutinin by bacteria cannot be analogous to the so-called adsorption of dissolved substances by charcoal, or of colouring matter by a fibre (Bordet, Biltz), because the velocity of the reaction in the former case is very great, equilibrium being reached in less than five minutes at 0° C. (Eisenberg and Volk), whereas, in the case of adsorption by charcoal or by a fibre, the process may be incomplete even after several days at the temperature of the room (Bordet, Hedin, etc.).

2. That the absorption of agglutinin by bacteria cannot be a chemical combination in the usual sense, unless it should be a very highly dissociable one, because, even accepting a high degree of dissociation, the fraction of agglutinin fixed (C) should increase to a limit value with increasing

concentration of the amount of agglutinin left (B) in the fluid after absorption has taken place, and with increase of the total amount of agglutinin originally present (T), yet no such limit can be observed in the experiments of Eisenberg and Volk on the absorption of agglutinin by bacteria, or those of Morgenroth and Arrhenius on the absorption of immune body by red corpuscles.

3. That the absorption of agglutinin by bacteria must be in nature different to the so-called adsorption processes, since in the latter n is generally found to be small (*e.g.* in the case of charcoal 0·25, Schmidt), while in the case of bacteria and agglutinin the value of n is from $\frac{2}{3}$ to 1.

4. That the accordance between the observed values and those calculated by the formula $C = kB^n$ (where $n = \frac{2}{3}$) is as good as could be expected in experiments of this kind, owing to the great difficulties in the technique.

Having thus briefly stated the arguments brought forward by Arrhenius in support of his view of the equilibria in the absorption processes of agglutinin by bacteria, we shall go on to our own experiments.

In deciding whether any such given formula offers a correct summarisation of the experimental facts upon which it is based, it is of the greatest importance that the experiments should be very numerous. It is no less important that the experiments should cover a wide range of concentrations. If these precautions are omitted, small deviations from the calculated values will easily be misinterpreted as experimental errors, when in point of fact they are periodical variations occurring with the utmost regularity. Now one of us (G. D.) had proved, in 1904, that the interaction between Coli agglutinin and the filtrate of old Coli culture ("toxin") did not follow the partition law $C = kB^n$ given by Arrhenius, which one might expect it to do if that law actually governed the combination of agglutinin with the specific substance in the bacteria. In addition, it seemed probable from the experiments of Eisenberg and Volk, and Morgenroth and Arrhenius, that periodical variations between values calculated according to this formula and those observed experimentally would occur. Accordingly, our own experiments were designed in such a way and given such a range as to minimise the likelihood that important differences such as these would be missed.

The technique employed was in all detail the same as that already described in our paper on the Velocity of Reaction in the Absorption of Specific Agglutinins by Bacteria, etc.* In the following experiments, coli and typhoid sera of different strengths and age, obtained from various animals immunised with different strains of bacteria, were used. Bacteria or bacterial filtrates ("toxin"), homologous to the serum taken for the experiment, were always

* P. 168, *supra*.

used, but in the case of a polyvalent serum only one race of the homologous bacteria was tried, the standardised test emulsion being in every case made of the same strain of bacteria as had been used for absorbing the agglutinins.

In every experiment, the volume of the bacterial emulsion was kept constant (4 c.c.), and allowed to act on a constant volume (4 c.c.) of the agglutinating serum in various dilutions. If volumes other than these were used, the fact will be found stated in the tables appended to this paper.

After mixing the bacterial emulsion, to be used for absorption, with the various dilutions of serum to be acted on, the tubes were immediately shaken, corked, and placed in a water bath at 37° C. for two hours, being shaken up again at the end of each hour. The tubes were then centrifugalised, and the supernatant fluid tested for the strength of its agglutinin content as described in the previous paper.

In some cases it was of importance to estimate a quantity of agglutinin less than one unit, and this was easily effected by substituting a part of the saline in each tube in the titration by a constant known fraction of a unit of agglutinin, obtained by dilution of the original serum, without altering the total volume in the tube. This method enabled us to determine quantities as small as nearly 0·4 unit. All values of agglutinin are expressed in arbitrary units as explained in the former paper.

Throughout our paper the following terms are used:—

T = the total number of units which the given dilution of serum contained before absorption.

B = the number of units left free in the supernatant fluid after absorption has taken place.

C = T minus B, *i.e.* the number of units removed from the original solution by the absorbing matter.

From the following description of our experiments, it will be clearly seen that our results are absolutely contradictory to the statements of Arrhenius on nearly every point, and we will therefore discuss in the light of our experiments each of the arguments summarised above and brought forward by that gifted chemist and mathematician in support of his theory that the interaction of bacteria and agglutinin can be expressed by the formula $C = kB^n$.

1. In another paper* we have fully proved that in the absorption of agglutinin by bacteria a considerable time elapses before equilibrium is

* G. Dreyer and J. S. C. Douglas, "The Velocity of Reaction in the 'Absorption' of Specific Agglutinins by Bacteria, and in the 'Adsorption' of Agglutinins, Trypsin, and Sulphuric Acid by Animal Charcoal," *supra*, p. 168.

reached. Thus we cannot accept the statement made by Eisenberg and Volk, and accepted by Arrhenius as being "in good agreement" with his theory, that the reaction has reached equilibrium "in less than five minutes even at 0° C."

2. In view of the statement by Arrhenius, based on the figures of Eisenberg and Volk for the absorption of agglutinin by bacteria and his own figures for the absorption of immune body obtained in conjunction with Morgenroth, that no limit value for C with increasing concentration of B can be observed, the following observations we have made are of great interest.

In Experiments 6, 8, 10, 11, 12 (Tables VII, IX, XI, XII, XIII) it will be seen that there is a great tendency for C to reach a limit value, in spite of an increase in the concentration of T, the total amount of agglutinins originally present; thus in Experiment 8, for example, it is seen that C remains practically the same, although T increases from 1845 to 2882, actually being 1475 and 1477. These experimental observations cannot be brought into agreement with Arrhenius' statement.

That this was not the peculiarity of a single stock of bacteria and its homologous serum is proved by the fact that Experiments 6 and 8 were carried out with a polyvalent serum, Experiment 10 with a different serum, and Experiments 11 and 12 with yet a third variety, as is detailed in the tables.

Further, it can be seen that this phenomenon does not depend on the agglutinating strength of the serum, nor on the amount absorbed, since in Experiment 8 the maximum absorption is 1477 units out of 2882, whilst in Experiment 12 the maximum absorption is only 147 out of the 732.

The point at which this limit value will be reached is dependent, not on the actual amount of agglutinin units present in the serum, but on a number of conditions, such as the amount of bacterial emulsion, the actual dilution of the serum, etc. Thus, if for instance a limit value is reached at a given concentration of the serum by treating it with a number of bacilli $10\ x$, the limit value would be reached at a lower concentration if the serum was treated with only x, and at a higher one if with $100\ x$, as is demonstrated by Experiments 6 and 8, and again in Experiments 11 and 12.

Therefore one of the main pillars in support of Arrhenius' theory on the absorption of agglutinins by bacteria falls to the ground because a continued increase in the size of C is an absolute necessity for the application of his formula $C = kB^n$, and thus for his explanation of the nature of the phenomenon.

Passing now to Experiments 2, 5, 7, 9, 14 (Tables III, VI, VIII, X, XV),

we see that with a constant amount of bacteria and an increase in the total amount of agglutinin present, C not only reaches a limit value at a certain concentration, but after this point has been reached actually decreases whilst the total amount of agglutinin present further increases, until at a given concentration no diminution in the agglutinin content of the serum can be traced as a result of its treatment with the bacterial emulsion; indeed, there seems in some cases even to be a tendency to a slight increase in the agglutinating strength of the serum, though not, in this series of experiments, of such degree as to be beyond the range of experimental error.

It is difficult to say how this phenomenon is to be explained, but, that it cannot be a question of the presence of so-called "agglutinoids" (Eisenberg and Volk) in the serum, the existence of which had been rendered most unlikely by the previous experiments of Dreyer and Jex-Blake, is clearly seen from the following fact.

If a serum is first treated with great quantities of bacteria it is found by afterwards using it for absorption experiments that not only is the attainment of a limit value not prevented, but that even the decrease in the actual size of C with increasing concentration of T is still evident (Experiment 9, Table X). This, however, could not occur if, as is said to be the case, the agglutinoids have a greater avidity for bacteria than the agglutinins have. A previous treatment of the serum by bacteria would free it from all, or the greater part of, such agglutinoids, so that the limiting value for C found after they have presumably been greatly diminished or got rid of cannot in any way be attributed to them.

The correctness of the statement that this phenomenon is not due to "agglutinoids" will be further proved by the absorption experiments undertaken with fresh agglutinating serum in both a heated and an unheated condition, referred to later in this paper.

In our opinion the phenomenon of a decrease in the value of C with increasing concentration of the serum is most likely caused by some obscure alterations in the surface tension due to change in the concentration of the albumen or of the different salts, or in the viscosity of the fluid, etc.

Such a limit value in C, and even an actual decrease in the size of C, may be reached, not only in the absorption processes of agglutinin by their own homologous bacteria, but may also occur if the agglutinin is acted on by non-specific bacteria, as, for example, a Coli agglutinin by an emulsion of typhoid bacilli (see Experiment 2, Table III).

We have also obtained similar results by treating an agglutinin in various dilutions with constant amount of animal charcoal, as will be described

in full in a later paper dealing with that subject,* and in addition we have been able to prove from Bayliss' figures dealing with the adsorption of Congo red by filter paper that the adsorbed amount C not only reaches a maximum, but even decreases subsequently, a fact to which Bayliss himself has drawn no attention.

3. That Arrhenius has no justification for drawing conclusions as to the nature of the interaction between bacilli and the homologous agglutinin from the size of the exponent n in his equation $C = kB^n$, a point on which he lays great stress as a means of distinguishing it from the adsorption processes, is clearly shown by our experiments.

Before dealing with the size of n and k it is worth while mentioning that if this formula were applicable, the line obtained by plotting out the logarithms of the values of B as ordinates, and of C as abscissæ, would be a straight one. How far this is from being the case can be seen from every single experiment recorded in this paper, and is further evident from the curve herein published. By plotting out the logarithms of the values of B and C in this way it is possible to determine n and k.

From Experiment 14 and curve, fig. 1, where the logarithms for values of

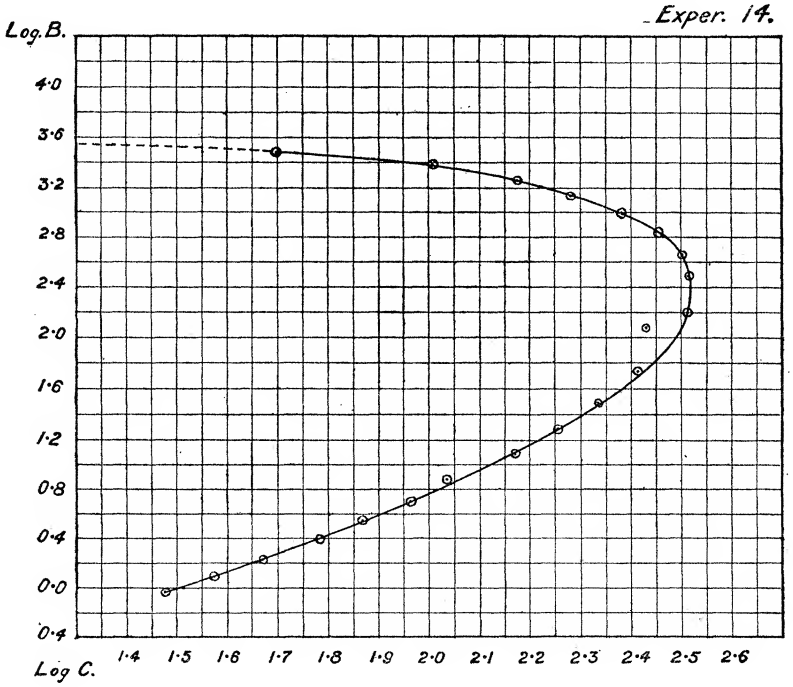

FIG. 1.

* A preliminary communication on this subject was given at the January meeting the Pathological Society of Great Britain and Ireland, 1909.

Table I.

	Experiment 7.					Experiment 14.					
	T.	B.	C.	"n" (when $k = 15{\cdot}74$).	"k" (when $n = 0{\cdot}8834$).		T.	B.	C.	"n" (when $k = 32{\cdot}0$).	"k" (when $n = 0{\cdot}7161$).
1 ...	2750	2750	0	—	—	1 ...	4850	4850	0	—	—
2 ...	2200	2170	30	0·0814	0·0339	2 ...	3880	3880	0	—	—
3 ...	1760	1600	160	0·3143	0·2363	3 ...	3100	3050	50	0·0556	0·1599
4 ...	1403	1140	263	0·4000	0·5242	4 ...	2475	2373	102	0·1491	0·3904
5 ...	1128	795	333	0·4569	0·9124	5 ...	1990	1840	150	0·2054	0·6990
6 ...	907·5	556	351·5	0·4913	1·321	6 ...	1600	1410	190	0·2456	1·0560
7 ...	715	354	361	0·5338	2·021	7 ...	1260	1020	240	0·2908	1·682
8 ...	550	196	354	0·5903	3·357	8 ...	970	684	286	0·3355	2·668
9 ...	440	108	332	0·6511	5·307	9 ...	776	457	319	0·3754	3·972
10 ...	357·5	60·5	297	0·7160	7·921	10 ...	631	303	328	0·4073	5·483
11 ...	275	34·4	240·6	0·7707	10·56	11 ...	485	160	325	0·4567	8·580
12 ...	220	22·8	197·2	0·8084	12·46	12 ...	388	118	270	0·4470	8·865
13 ...	176	16·5	159·5	0·8260	13·41	13 ...	310	50·6	259·4	0·5332	15·62
14 ...	140·3	11·8	128·5	0·8506	14·52	14 ...	247·5	31	216·5	0·5567	18·51
15 ...	112·8	9	103·8	0·8584	14·90	15 ...	199	18·9	180·1	0·5877	21·95
16 ...	90·75	6·7	84·05	0·8806	15·69	16 ...	160	11·8	148·2	0·6210	25·31
17 ...	71·5	5·1	66·4	0·8834	15·74	17 ...	126	7·7	118·3	0·6404	27·43
18 ...	55	3·8	51·2	0·8834	15·74	18 ...	97	5	92	0·6560	29·05
						19 ...	77·6	3·6	74	0·6543	29·56
						20 ...	63·1	2·5	60·6	0·6969	31·45
						21 ...	48·5	1·7	46·8	0·7161	32·00
						22 ...	38·8	1·25	37·55	0·7161	32·00
						23 ...	31	0·95	30·05	1·2286	31·18

B and C have been plotted out, it is clearly seen that it is quite impossible to draw a straight line through more than two consecutive points, but that the line drawn through all the points is actually a curve deviating more and more from the straight line, and away from the abscissa as the concentration of the agglutinating serum is increasing. This is caused by the fact that from the very outset C does not increase as rapidly as it should according to Arrhenius' formula. Also it is seen from this and the other experiments how impossible it is to find a constant exponent n if k has to be a constant, or *vice versâ*, as demanded by Arrhenius' formula $C = kB^n$. This is well shown in the following table of figures calculated from Experiments 7 and 14 (Table I).

On the other hand, it is possible in experiments which only cover a short range, to draw a line which will allow the determination of an n and k which will give calculated values for B approximating to those observed—but with a regular periodicity in the deviation.

A similar periodicity in the change of value of k is also to be traced in the experiments of Eisenberg and Volk, and of Arrhenius and Morgenroth, though with some difficulty owing to the great distance between the points experimentally determined.

In the different tables (II to XV) we have calculated from point to point the exponent n, and it exhibits enormous variations in size according to the region examined in each experiment.

In Experiment 14 (Table XV), for example, we find that n will have a value of about 0·8 in the weakest concentration of the serum. From this point it will gradually decrease in size to zero, and then, becoming negative in sign, increase towards infinity. That this behaviour does not depend on the strain of bacillus or the brand of agglutinating serum is proved by any or all of the experiments undertaken with various bacteria and their homologous agglutinin (see Experiments 1, 2, and 5 to 14, Tables II, III, and VI to XV).

Experiment 5 (Table VI) proves that the variation in n is not caused by the presence of the quantities of formalin added to the emulsions.

To make it clear that this deviation could not be explained by the presence of "agglutinoids" in the serum, or substances analogous to such bodies in the bacteria, experiments which gave absolutely similar results were undertaken with heated serum and unheated Coli bacilli filtrate ("toxin") (Experiments 3 and 4, Tables IV and V), and with heated bacilli and unheated serum (Experiments 1 and 6, Tables II and VII). Now, if any such bodies had been formed by the action of heat, the deviation from a straight line of the curve obtained by plotting out log. B and log. C should become more marked. That this, however, is not the case, is readily seen.

Experiment 7 (Table VIII), in which the serum before use had been treated with bacteria to remove such bodies if they were present, offers additional proof on this point.

It is very interesting to note that it is not at present possible to distinguish between the action of the Coli bacilli filtrate ("toxin") and that of the bacteria themselves, on agglutinin; this is seen from Experiments 2 and 3 (Tables III and IV). Therefore the supposition expressed by one of us (G. D.) on an earlier occasion that, as the partition law of Arrhenius did not hold good for the interaction of such "toxin" and agglutinin the same would most likely be found to be the case if the interaction of bacteria and agglutinin were examined, was correct.

A further point of interest is that in spite of the partition law not holding good in the case of "toxin" and agglutinin, the above formula will, nevertheless, if used for calculation, give a better agreement between calculated and experimental figures in this case than in that of bacteria and agglutinin.

Comparing together the absorptions of agglutinin from heated serum and from unheated serum, and plotting out the values log. B and log. C, it appears that the deviation from the straight line is less in the case of the heated serum. The higher the temperature, within certain ranges, to which the serum is heated, the more constant will n be found within corresponding ranges of concentration of serum, and the nearer to a straight line will be the curve obtained. Thus in Experiment 4 (Table V), where the serum was heated to 70° C., and there was an increase in concentration from about 4 to 60 units, nothing better than a straight line can be drawn, giving an n of about 0·73, and a k of about 1·0, while in Experiment 3 (Table IV), where the serum is only heated to 60° C., n varies from about 1·1 to 0·3.

From the whole series of experiments it is clear that one is not justified from the size of n in forming a conclusion (as done by Arrhenius) as to the nature of the interaction, because n may have any size from near one to minus infinity in the same experiment, depending only on the concentration of the serum. This is the more the case since we have found variations in the size of n of very much the same kind in the "adsorption" of agglutinins and other bodies by charcoal, as we shall describe in a later paper.*

It is further seen from Experiments 1 to 14 (Tables II to XV) that no great stress can be laid on the actual size of n or k, since n is not alone, or even mainly, dependent upon the kind of absorbing matter or substance

* A preliminary communication on this subject was given at the January meeting of the Pathological Society of Great Britain and Ireland, 1909.

absorbed, but on the varying amounts of both, the presence of albumen, dilution of serum, presence of salts, temperature, time of reaction, and other variable factors.

4. Turning to the fourth point brought forward by Arrhenius, that the agreement is satisfactory between observed figures and those calculated according to his formula $C = kB^n$ and published in his papers, we consider that the accordance between his figures is by no means good. Arrhenius himself regards the deviations he finds as entirely due to experimental error, because he was informed by Eisenberg and Volk that such great variations were quite possible in the technique that they had used. To us it is clear that, leaving alone the great experimental error, all the experiments calculated by Arrhenius show a certain periodicity in the increase and decrease of k in the same direction as found in our own experiments, but not taken into consideration by him. Our own results show that, even if we chose the best possible n, B calculated and B observed will only agree approximately within a certain small range. Beyond these limits the values for B observed will be much bigger than for B calculated, a natural result of what has been stated previously, that C does not increase so fast as it should if Arrhenius' formula $C = kB^n$ were correct.

As we have proved beyond dispute the great and regular variations occurring in the "constants" n and k of Arrhenius' formula $C = kB^n$ when applied to the absorption of agglutinin by bacteria, we record no figures calculated according to that expression, since it is now obvious how absolute must be the disagreement between the observed values of B and those thus calculated.

At a later date it is our hope to deal with the mathematical considerations arising in connection with the figures in this paper.

Table II.—Experiment 1. October 26, 1904.

5 c.c. of typhoid serum (T 29). 5 c.c. of 1·5/1 N emulsion of typhoid bacilli without formalin. Y, bacilli unheated; Z, bacilli previously heated to 60° C. for 1 hour. Actual strength of serum in tube 1 = 50 per cent.

		Y.			Z.		
	T.	B.	C.	*n.*	B.	C.	*n.*
1	111·2	76·9	34·3		78·0	33·2	
				0·186			0·297
2	44·4	18·2	26·2		21·7	22·7	
				0·57			0·704
3	22·2	7·0	15·2		9·5	12·7	
				0·876			1·004
4	11·1	3·22	7·88		4·76	6·34	
				0·798			1·31
5	5·55	1·43	4·1		2·63	2·92	
							0·870
6	2·78	<1·0	—		1·25	1·53	
7	1·39	<1·0	—		<1·0	—	

Table III.—Experiment 2. November 10, 1904.

5 c.c. *Coli lab.* serum (*x*) 61, 1/1. Y, 10/1 N *Coli lab.* bacilli without formalin. Z, 10/1 N typhoid bacilli without formalin. Actual strength of serum in tube 1 = 50 per cent.

		Y.			Z.		
	T.	B.	C.	*n.*	B.	C.	*n.*
1	2000	1970	30		2000	0	
				2·16			—
2	1000	775	225		911	89	
				0·154			0·28
3	500	305	195		428	72	
				0·435			0·737
4	250	120	130		211	39	
				0·571			0·956
5	125	48	77		105	20	
				0·508			0·839
6	62·5	17	45·5		51·5	11·0	
				0·551			0·881
7	31·3	5·9	25·4		25·4	5·9	
							0·91
8	15·6	1·0	—		12·5	3·1	
							0·91
9	7·8	1·0	—		6·17	1·63	

Table IV.—Experiment 3. February 9, 1905.

1 c.c. of Coli serum (*x*) 61, unheated (Y) and heated undiluted to 60° C. for 1 hour (Z). 9 c.c. Coli bouillon culture filtrate ("Toxin" 21), grown 21 days at 37° C. before filtration. Actual strength of serum in tube 1 = 10 per cent.

	Y.				Z.			
	T.	B.	C.	*n.*	T.	B.	C.	*n.*
1	880	775	105		708	490	218	
				0·151				0·535
2	440	347	93		354	214	140	
				0·213				0·569
3	220	143	77		177	91	86	
				0·303				0·57
4	110	53	57		88·5	37	51·5	
				0·517				0·651
5	55	20·3	34·7		44·3	15·3	29	
				0·865				0·894
6	27·5	9·5	18		22·1	7·24	14·86	
				1·03				1·255
7	13·8	4·76	9·04		11·06	4·0	7·06	
				1·11				0·895
8	6·9	2·46	4·44		5·53	1·9	3·63	
				1·17				0·995
9	3·4	1·3	2·1		2·77	0·95	1·82	

Table V.—Experiment 4. February 13, 1905.

1 c.c. of Coli serum (*x*) 61, diluted 1/5 and then heated to 70° C. for 1 hour. 9 c.c. of Coli ("Toxin" 21) (see Exp. 3, Table IV). Actual strength of serum in tube 1 = 2 per cent.

	T.	B.	C.	*n.*
1	60·5	41·7	18·8	
				0·789
2	30·25	19·8	10·45	
				0·738
3	15·13	9·2	5·93	
				0·725
4	7·56	4·2	3·36	
				0·732
5	3·78	1·9	1·88	
6	1·89	<1·0	—	

Table VI.—Experiment 5. February 18, 1905.

5 c.c. of Coli serum (x) 61, diluted 1/2. 5 c.c. of 10/1 N Coli culture without formalin. Actual strength of serum in tube 1 = 25 per cent.

	T.	B.	C.	n.
1	1820	1820	0	
2	910	810	100	
				−0·24
3	455	331	124	
				0·121
4	227·5	118	109·5	
				0·205
5	113·8	30·7	83·1	
				0·215
6	56·9	3·82	53·08	
7	28·4	<1·0		
8	14·2	<1·0		

Table VII.—Experiment 6. March 12, 1907.

4 c.c. Coli serum, Forsög's polyvalent, diluted 1/5. 4 c.c. 10/6 N emulsion of Coli Aunsögaard, February 23, 1907. Y, unheated; Z, previously heated to 100° C. for 5 minutes. Actual strength of serum in tube 1 = 10 per cent.

		Y.			Z.		
	T.	B.	C.	n.	B.	C.	n.
1	530	159	371		162	368	
				0·0768			0·0734
2	432	80	352		82	350	
				0·37			0·397
3	349	51	298		53·5	295·5	
				0·567			0·750
4	283	36·5	246·5		41	242	
				0·595			0·923
5	231	27·5	203·5		33·0	198·0	
				1·17			0·95
6	188	23	165		26·6	161·4	
				0·725			1·083
7	153·5	17·6	135·9		22	131·5	
				0·905			1·048
8	124·5	14	110·5		18	106·5	
				0·594			0·641
9	100·5	10	90·5		13·2	87·3	

Table VIII.—Experiment 7. March 20, 1907.

4 c.c. of Coli serum, Forsög's polyvalent, 1/1. 4 c.c. of 10/9 N Coli Aunsögaard emulsion, February 23, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n*.
1.....	2750	2750	0	
2.....	2200	2170	30	−5·49
3.....	1760	1600	160	−1·467
4.....	1403	1140	263	−0·649
5.....	1128	795	333	−0·145
6.....	907·5	556	351·5	−0·0591
7.....	715	354	361	0·0328
8.....	550	196	354	0·109
9.....	440	108	332	0·192
10.....	357·5	60·5	297	0·374
11.....	275	34·4	240·6	0·478
12.....	220	22·8	197·2	0·656
13.....	176	16·5	159·5	0·646
14.....	140·3	11·8	128·5	0·787
15.....	112·8	9·0	103·8	0·715
16.....	90·75	6·7	84·05	0·864
17.....	71·5	5·1	66·4	0·883
18.....	55	3·8	51·2	

Table IX.—Experiment 8. March 22, 1907.

4 c.c. of Coli serum, Forsög's polyvalent, 1/1. 4 c.c. of 10/2 N Coli Aunsögaard emulsion, March 15, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n*.
1	2882	1405	1477	
				0·00555
2	1845	370	1475	
				0·372
3	1469	231	1238	
				0·680
4	951	127	824	
				0·831
5	749	96	653	
				0·957
6	461	58	403	

Table X.—Experiment 9. April 12, 1907.

4 c.c. of an old *Coli lab.* serum (goat), previously diluted and treated with an emulsion of bacilli. 4 c.c. of 10 N *Coli lab.* emulsion, April 8, 1907. Actual strength of serum in tube 1 not known.

	T.	B.	C.	*n*.
1	411	222	189	
				−0·0826
2	339	143	196	
				0·0106
3	253	58·8	194·2	
				0·133
4	209	30·8	178·2	
				0·224
5	168	15·4	152·6	
				0·296
6	136	8·4	127·6	
				0·351
7	107	4·5	102·5	
				0·461
8	82·2	2·6	79·6	
				0·509
9	65·8	1·7	64·1	
10	53·4	<1·0		
11	41·1	<1·0		
12	33·9	<1·0		

Table XI.—Experiment 10. April 26, 1907.

4 c.c. of Coli Kringelgaard (horse) serum, 1/1. 4 c.c. of 20 N Coli Kringelgaard emulsion, April 24, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n.*
1	961	363	598	
				0·0349
2	768	184	584	
				0·0402
3	615	67	548	
				0·136
4	490	21·3	468·7	
				0·204
5	394	8·3	386·7	
				0·29
6	317	4·0	313	
7	250	>2	248	0·348 (from Nos. 6 and 8)
8	194·5	1·0	193·5	
9	153·8	<1·0		
10	124·9	<1·0		

Table XII.—Experiment 11. October 14, 1908.

4 c.c. of *Coli lab.* (goat) serum, June 16, 1907, 1/1. 4 c.c. of 30 N *Coli lab.* emulsion, June 26, 1907. Actual strength in tube 1 = 50 per cent.

	T.	B.	C.	*n.*
1.....	805	209	596	
				0·235
2.....	643	120	523	
				0·215
3.....	515	61·7	453·3	
				0·222
4.....	411	28·7	382·3	
				0·274
5.....	330	14·3	315·7	
				0·324
6.....	266	7·7	258·3	
				0·592
7.....	209	4·76	194·24	
				0·482
8.....	161	3·1	157·9	
				0·71
9.....	128·8	2·27	126·53	
				0·895
10.....	104·6	1·8	102·8	
				0·801
11.....	80·5	1·3	79·2	
				0·666
12.....	64·3	0·93	63·37	
				0·866
13.....	51·5	0·72	50·78	
				0·904
14.....	41·1	0·55	40·55	
				0·9
15.....	33	0·44	32·56	
16.....	26·6	<0·31		
17.....	20·9	<0·31		
18.....	16·1	<0·31		

Table XIII.—Experiment 12. October 19, 1908.

4 c.c. of *Coli lab.* (goat) serum, June 16, 1907, 1/1. 4 c.c. of 6·67/1 N *Coli lab.* emulsion, June 26, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n.*
1.....	723	585	147	
				0·125
2.....	586	444	142	
				0·122
3.....	468	331	137	
				0·131
4.....	373·5	242	131·5	
				0·149
5.....	300·3	175	125·3	
				0·131
6.....	241·5	122	119·5	
				0·146
7.....	193	80·5	112·5	
				0·190
8.....	146·3	45·4	100·9	
				0·183
9.....	117·1	26	91·1	
				0·301
10.....	95·2	16·2	79·0	
				0·246
11.....	73·2	7·6	65·6	
				0·272
12.....	58·6	3·9	54·7	
				0·5
13.....	37·35	1·66	35·69	
				0·885
14.....	30·03	1·3	28·73	
				0·615
15.....	24·15	0·92	23·23	
				0·696
16.....	19·3	0·67	18·63	
				0·283
17.....	16·63	0·41	16·22	

Table XIV.—Experiment 13. December 17, 1908.

4 c.c. of *Coli lab.* (goat) serum, June 16, 1907, 13/100. 4 c.c. of 12·5/1 N *Coli lab.* emulsion. Actual strength of serum in tube 1 = 6·5 per cent.

	T.	B.	C.	*n.*
1	68·8	8·33	60·47	
				0·302
2	53·0	4·12	48·88	
				0·402
3	42·4	2·49	39·91	
				0·625
4	33·9	1·76	32·14	
				0·848
5	27·0	1·35	25·65	
				0·898
6	21·1	1·05	20·05	
				1·049
7	17·5	0·83	16·67	

Table XV.—Experiment 14. June 14, 1909.

4 c.c. of *Coli lab.* (goat) serum, February 26, 1909, 1/1. 4 c.c. of 40 N *Coli lab.* emulsion, May 29, 1909. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n.*
1	4850	4850	0	
2	3880	3880	0	
3	3100	3050	50	
				−2·84
4	2475	2373	102	
				−1·52
5	1990	1840	150	
				−0·889
6	1600	1410	190	
				−0·722
7	1260	1020	240	
				−0·466
8	970	684	286	
				−0·2710
9	776	457	319	
				−0·0677
10	631	303	328	
				0·0144
11	485	160	325	
				0·196 (from Nos. 11 to 13)
12	388	118	270	
13	310	50·6	259·4	
				0·368
14	247·5	31	216·5	
				0·372
15	199	18·9	180·1	
				0·413
16	160	11·8	148·2	
				0·528
17	126	7·7	118·3	
				0·583
18	97	5·0	92·0	
				0·662
19	77·6	3·6	74·0	
				0·548
20	63·1	2·5	60·6	
				0·671
21	48·5	1·7	46·8	
				0·716
22	38·8	1·25	37·55	
				0·811
23	31·0	0·95	30·05	
24	24·75	<1·0		
25	19·9	<1·0		
26	16·0	<1·0		
27	12·6	<1·0		

Conclusions.

1. When an agglutinating serum in different concentrations is treated with constant amounts of bacteria, the quantity absorbed C may not

only increase to a limit value but may, when this point is passed, even decrease to zero when the concentration of the serum is further increased.

2. It is impossible, from the greater or smaller size of the exponent "n" in the formula $C = kB^n$, to determine whether in the case of agglutinin we have to deal with an absorption or an adsorption process, as in both cases "n" may vary within nearly the same ranges.

3. The formula $C = kB^n$, proposed by Arrhenius to express the absorption of agglutinin by bacteria, as being a special example of the Guldberg and Waage law of chemical mass action, does not hold good either in the case of absorption of agglutinin by bacteria, or of the neutralisation of agglutinin by homologous bacterial filtrate ("toxin").

4. The combination of agglutinin and bacteria is, therefore, not such a simple process as anticipated by Arrhenius, but is very possibly complex, and not improbably of the same nature as the interaction of bacterial toxins and antitoxins.

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Observations on the Rate of Action of Drugs (Alcohol, Chloroform, Quinine, Aconitine) upon Muscle as a Function of Temperature.

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Very scanty information exists as regards the effect of temperature upon the activity of drugs. Brunton* says:—"That the action of veratrine and of barium on muscle is very much altered by heat and cold. Many, if not all, muscular poisons act more quickly with increased temperature."

According to the same author, Humboldt† noticed that warmth increases the rapidity with which alcohol destroyed the irritability of a nerve and potassium sulphide that of a muscle. Brunton and Cash‡ showed that "up to a certain point heat increases the effect of veratria and cold lessens it." Waller took observations of the rates of action of alcohol, ether, and

* 'Pharmacology,' 1893, p. 45.

† 'Ueber die gereizte Muskel und Nervenfaser,' 1797, vol. 2, p. 218.

‡ 'Journ. of Physiol.,' 1883, vol. 4, p. 1.